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Microbiological contamination linked to implementation of good agricultural practices in the production of organic lettuce in Southern Brazil

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ABSTRACT

Interviews were conducted with the owners of three organic lettuce farms in the state of Rio Grande do Sul in southern Brazil using a standardized self-assessment questionnaire to ascertain the status of implementation of good agricultural practices and management systems in place. In addition, on each farm 132 samples (manure, field soil, water, workers' hands and equipment, lettuce seedlings, and crops) were collected during four visits throughout the lettuce crop production cycle and subjected to analysis for hygiene indicators (*Escherichia coli*, coliforms) and presence of *Salmonella* and *E. coli* O157. *E. coli* O157 was detected twice (in irrigation water and in rinse water) out of 27 analyzed water samples. *Salmonella* spp. was detected in one out of nine manure samples applied as organic fertilizer. In addition, generic *E. coli* was frequently present in numbers exceeding 10 cfu/g in manure, manured soil, and lettuce samples or more than 1 cfu/100 ml in water. No *E. coli* O157 was detected in any of the lettuce samples ($n = 36$), but *Salmonella* spp. was detected once in lettuce taken during the crop cycle 2 weeks before harvest. It was demonstrated that the combination of the self-assessment questionnaire and microbiological sampling and analysis could identify weak points in current organic farming practices in this region of southern Brazil. It was noted that manure composting was not adequately controlled and appropriate waiting times before application as an organic fertilizer to crop were not respected. Also the selection of the water source and the sanitary quality of the water used for irrigation were not under control. The washing step (with sanitizer) of lettuce crops at harvest, generally considered a potential reduction step for microbial contamination, was often not verified for its efficiency. This may detract from the sanitary quality of the produce and are risk factors for the introduction of pathogens in the lettuce sent to market. The study, combining interviews, sampling, and analysis, contributed to increasing the farmers' awareness of enteric pathogens as a food safety issue in leafy greens. Further communication and training on good agricultural practices are recommended to remediate the weak points identified in the current management system.

1. Introduction

The search for healthy, safe, and sustainable food production has increased the consumption of organic fresh produce. These products should be free of pesticide residue and other synthetic substances commonly used in conventional agriculture, such as inorganic

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soluble fertilizers (Aquino & Assis, 2007; Assis & Romeiro, 2002). At the same time that organic products have lowered risks related to chemical contamination, several investigations have raised concerns with reference to the microbiological quality of these foods (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Abreu, Junqueira, Peixoto, & Oliveira, 2010; Delaquis, Bach, & Dinu, 2007; Lotto, 2008; Oliveira, Ritter, Tondo, & Cardoso, 2012; Oliveira, Viñas, Usall, Anguera, & Abadias, 2012; Oliveira et al., 2010; Rezende & Farina, 2001). Among organic fresh produce, lettuce (*Lactuca sativa* L.) stands out due to its continuous availability on the market as well as its acceptability indistinctively of age or economic group of the population (Abreu et al., 2010; Cometti, Matias, Zonta, Mary, & Fernandes, 2004). However, lettuce might become the foremost means of microbiological contamination due to its imbricate leaves, which provide conditions for the survival and potential growth of microorganisms (Johannessen et al., 2004; Steele & Odumeru, 2004; Suslow et al., 2003). The microbiological contamination of lettuce is likely to occur at several steps of the production chain, whether the production system is organic, hydroponic, or conventional. For that reason, evaluation of the sanitary conditions of each production location is of the utmost importance. Pathogenic microorganisms have been frequently detected in soil, fertilizers, irrigation, and rinse waters (Ilic et al., 2012; Itohan, Peters, & Kolo, 2011; Machado, Bueno, Oliveira, & Moura, 2009; Mocelin & Figueiredo, 2009; Moretti & Mattos, 2006; Olaimat & Holley, 2012; Oliveira, Ritter et al., 2012; Oliveira et al., 2010; Oliveira, Viñas et al., 2012; Taban & Halkman, 2011; WHO, 2008).

According to Mogharbel and Masson (2005); Ilic et al. (2012) and many other studies, the reduction of contamination risks is directly linked to application of Good Agricultural Practices (GAP). GAPs are defined on an international level in the Codex Alimentarius Commission's code of practice for fresh fruits and vegetables (CAC/RCP 53-2003) (Codex, 2003). The code of practice concerns all activities in and around fields before, during, and after production and harvest (i.e., water quality, personal hygiene of the workers, manure composting, etc.) (CAC/RCP 53-2003). At production areas, irrigation and rinse waters have received attention, as they might be some of the major sources of microbial contamination. Irrigation and rinse waters might contain pathogenic bacteria such as *Salmonella* spp. and *Escherichia coli* O157:H7. Usually, irrigation and rinse waters are used without any previous treatment when obtained from rivers, streams, lakes, or wells adjacent to the cropping areas (Abreu et al., 2010; Ilic et al., 2012; Olaimat & Holley, 2012; Pacheco et al., 2002; Salem, Ouardani, Hassine, & Aouni, 2011). Microbiological contamination data for the production chain of organic lettuce in Brazil is not currently available. Therefore, the objectives of this study were to investigate the status of the implementation of good agricultural practices and management systems and the impact on microbial contamination in organic lettuce production in southern Brazil. Three organic farms were selected for a case study and were visited four times during the lettuce crop production cycle for interviews, using a standardized self-assessment questionnaire (Kirezieva, Jacxsens, Uyttendaele, Van Boekel, & Luning, 2013; Kirezieva, Nanyunja et al., 2013), observations and sampling in order to get insights about the variables that contribute to the microbiological contamination (i.e., coliforms, generic *E. coli*, *E. coli* O157 and *Salmonella* spp. in lettuce production were used).

2. Material and methods

2.1. Characteristics of organic lettuce growers

Three growers of organic lettuce participated in this study and were designated as Farm 1, 2, or 3. All of the growers were located in the rural area of Rio Grande do Sul, the southernmost state in Brazil, and they were selected because they represent typical organic farms of this region. These farms mainly produced organic lettuces and were operating in accordance with the Organism of Social Control (OSC) and with the Participative Organization of Organic Compliance (OPAC) of the southern region of Brazil. OPAC corresponds with certification bodies, accredited by the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA), who evaluate the farms' compliance with organic production standards. All farms claimed to be aware of and using Good Agricultural Practices (GAP). However, these practices were neither registered nor written down. All farms have open field production, and the organic lettuce seedlings for the three different farms originated from the same supplier. The fertilization system diverged in all three farms. At Farm 1, the fertilizer came from poultry manure, and at Farm 2, horse manure was the source of fertilizer for the lettuce fields. The grower at Farm 3 prepared his fertilizer by using only vegetable scraps. The composting time for each of these sources of organic matter differed and was usually in the range of 180 days, 90 days, or 60 days at Farms 1, 2, or 3, respectively.

The sprinkler irrigation system at Farm 1 was supplied with collected rainwater from a nearby pond. At Farm 2, a drip irrigation system was used. The water was pumped from a well into a storage tank. At Farm 3, the irrigation system was supplied by a pond close to the plantation area from which water was pumped into a storage tank, and the water was delivered through a hose to the lettuce fields.

2.2. Microbiological sampling plan

2.2.1. Sampling locations

A microbial sampling plan was developed, directed at identifying bottlenecks in the management of food hygiene and safety by sampling at critical sampling locations (CSLs). These sampling locations were selected based on the literature review of potential risk factors that contribute to the microbiological contamination of crops and lettuce in particular. For organic lettuce farms, 12 critical sampling locations were selected (Fig. 1), including the lettuce crop (or seedling at time of planting) and other sources of potential microbiological contamination, as identified in the literature reviews (Ilic et al., 2012; Olaimat & Holley, 2012) (i.e., soil, water, manure, food contact surfaces, or food handlers). Each farm was visited at the start of the crop cycle, two weeks before harvest, one week before harvest, and at harvest. This process was repeated per farm for three lettuce crop cycles (Fig. 1 and Table 1). Thus, three lettuce crop production cycles per farm were monitored. Sampling was conducted in the period from December 2011 until February 2012. The microbial sampling plan was set up to obtain a helicopter view on hygiene (*E. coli*, coliforms), and the safety (*Salmonella* spp., *E. coli* O157) level of the selected organic farms' production systems. The sampling plan was also set up in order to provide a supporting microbial data collection, in addition to observations and the outcome of the interviews with the farmers with a standardized self-assessment questionnaire (Kirezieva, Jacxsens et al., 2013; Kirezieva, Nanyunja et al., 2013).

2.2.2. Sampling method

For the sampling of the irrigation water sources at each farm, a 5-L sample was collected in a sterilized plastic bottle. The bottle was immersed upside down into the water source to a depth of 20–30 cm. The bottle was also filled while turned sideways and upwards in order to avoid superficial contamination.

For the sampling of irrigation water from the tap of the sprinklers or a drip, a sample of 5 L was collected into a sterilized plastic bottle. Before each collection, the irrigation water taps were

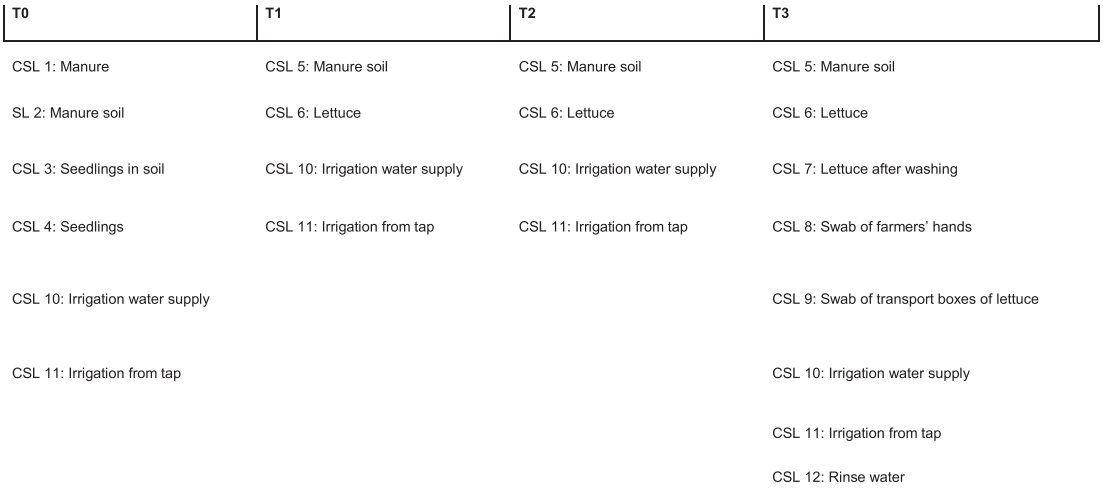

Fig. 1. Timeline and identification of selected critical sampling locations (CSL) in primary production of organic lettuce in Southern Brazil. T0: Start of the planting; T1: three weeks before harvest; T2: two weeks before harvest; T3: harvest.

disinfected with 70% ethyl alcohol. Then the taps were opened and after the water flowed for 60 s, the water was collected directly into the sterile bottle. The irrigation water samples were collected during each of the four visits to each of the three growers.

The lettuce plants were washed in tanks with potable water, supplied by the public water service department, water from a well, or water from a pond at Farm 1, 2, and 3, respectively. After the harvested lettuce was washed, 5 L of residual rinse water were collected into a sterilized plastic bottle. The rinse water was collected only during the last visit and after the harvesting and washing processes of the lettuce.

Soil samples were collected from a 30 cm^2 area around each sampled lettuce plant. Each soil sample consisted of 200 g of soil. Soil samples were collected during every visit to the growers. At each production area, three soil samples were taken and pooled to produce one single soil sample for every grower on every visit. This procedure resulted in a total of nine soil samples, analyzed along the production timeline of lettuce. The samples were placed in plastic bags and transported by car to the laboratory for subsequent microbiological analyses.

One single fertilizer sample was retrieved from every production area at the beginning of the production. A 200-g sample was withdrawn from the composting location, placed directly into sterile plastic bags and transported to the laboratory.

With the use of sterile plastic bags, 500 g of lettuce seedlings were collected before transplanting, as they were delivered by the suppliers. The lettuce seedlings were collected during the first visit to the growers.

The lettuce plants were cut just above the ground with a knife previously disinfected with 70% ethyl alcohol. The samples were placed directly into sterile plastic bags. From each producer, during each of the visits, nine samples of lettuce plants were collected randomly, according to a Z profile in the field. During the visit at harvest, nine samples of washed lettuce were also collected. At all of the sampling times, of these nine samples taken, three cut lettuce heads or washed lettuce were all placed in a sterile bag to obtain 3 pooled samples for analysis.

To determine the microbial load and hygiene of workers' hands, three swab samples were collected on each farm. To determine the microbial load and hygiene of the lettuce transport boxes, three swab samples were also collected on each farm. These samples were collected from three different workers' hands, only during the last visit amid the harvesting activities.

For the sampling of the lettuce transport boxes, an area of 50 cm^2 was drawn with a previously disinfected wire mold. One sample was collected from each box, amounting to three samples from each grower, prior to using the boxes to pack the harvested lettuce. Before the sampling, the swabs were moistened in sterile 0.1% peptone water and rubbed in three different directions in the delimited area on the hands or the transport boxes. Subsequently, the swabs were placed in test tubes that contained sterile 0.1% peptone water.

All of the collected samples were transported by car in thermal boxes, under refrigeration (7 °C) in less than 1 h to the Food Microbiology and Food Control Laboratory of the Institute of Food Science and Technology at ICTA/UFRGS for further analyses.

2.2.3. Microbiological parameters and methods of analysis

The analyses and microbiological parameters of each sampling location are presented in Table 1. Coliforms, *E. coli*, and *Enterococcus* spp. were used as hygiene indicator organisms. The total coliforms were considered as indicators for the overall quality of the sanitation for the samples. *Enterococcus* and *E. coli* were considered as fecal contamination indicators of the samples. *E. coli* O157 and *Salmonella* spp. were analyzed as enteric pathogens. Microbial analyses were implemented by using the standard methodologies described in Table 1.

Salmonella spp. was determined according to the methodology described in ISO 6579:2002 (ISO, 2002). Characteristic colonies of *Salmonella* spp. were confirmed by biochemical tests (API 30E, BioMerieux). Serological testing was performed by using polyvalent serum anti O (Probac do Brasil). Moreover, isolates, identified as *Salmonella*, were sent to the reference Laboratory of *Enterobacteriaceae* at the Bacteriology Department of the Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (FIOCRUZ) in order to be serotyped.

The analyses of total coliforms and *E. coli* were made with samples of lettuce seedlings, crops, manure, and field soil samples. Ten grams of the samples were placed in 90 ml 0.1% peptone water. The samples were homogenized in a stomacher (Seward) for 30 s. Decimal dilutions were prepared, and triplicate samples of 1 ml were placed on Petrifilm™ plates and incubated for 24 ± 2 h at 37 ± 1 °C.

At the laboratory, water samples of 100 ml or 25 ml were retrieved from the 5-L samples collected at the production fields to be analyzed as described below. To determine coliforms and *E. coli* of irrigation waters, the Most Probable Number (MPN) method,

Table 1
Descriptions of critical sampling locations (CSL), samples, time of samplings, microbiological parameters, microbiological methodologies, results interpretations, and references for the interpretation of the developed risk-based sampling plan. This sampling plan was conducted three times for each involved farm.

CSL	Description	Samples	Time	Microbiological parameters	Methodology	Interpretation of the results[a]	References
1	Manure	3 samples	T0	*E. coli*/coliforms	ISO 21528-2:2004	1.000 NMP/g	*MAPA/IN n°46. (2011)*
				E. coli O157:H7	and AOAC (1998)	A/25 g	ND
				Salmonella spp.	ISO 16654:2001	A/25 g	*MAPA/IN n°46. (2011)*
					ISO 6579:2002		
2	Manured soil	3 samples → 3 × 3 pooled	T0	*E. coli*/coliforms	ISO 21528-2:2004	1.000 NMP/g	*MAPA/IN n°46. (2011)*
				E. coli O157:H7	and AOAC (1998)	A/25 g	ND
				Salmonella spp.	ISO 16654:2001	A/25 g	*MAPA/IN n°46. (2011)*
					ISO 6579:2002		
3	Seedlings in soil	1 sample → 1 × 3 pooled	T0	*E. coli*/coliforms	ISO 21528-2:2004	10^2	*RDC n°12 (2001)*
				E. coli O157:H7	and AOAC (1998)	A/25 g	ND
				Salmonella spp.	ISO 16654:2001	A/25 g	*RDC n°12 (2001)*
					ISO 6579:2002		
4	Seedling	1 sample	T0	*E. coli*/coliforms	ISO 21528-2:2004	10^2	*RDC n°12 (2001)*
					and AOAC (1998)		
5	Manured soil	3 samples → 3 × 3 pooled	T1	*E. coli*/coliforms	ISO 21528-2:2004	10^2	*MAPA/IN n°46. (2011)*
			T2	*E. coli* O157:H7	and AOAC (1998)	A/25 g	ND
			T3	*Salmonella* spp.	ISO 16654:2001	A/25 g	*MAPA/IN n°46. (2011)*
					ISO 6579:2002		
6	Lettuce	3 samples → 3 × 3 pooled	T1	*E. coli*/coliforms	ISO 21528-2:2004	10^2	*RDC n°12 (2001)*
			T2	*E. coli* O157:H7	and AOAC (1998)	A/25 g	ND
			T3	*Salmonella* spp.	ISO 16654:2001	A/25 g	*RDC n°12 (2001)*
					ISO 6579:2002		
7	Lettuce after washing	3 samples → 3 × 3 pooled	T3	*E. coli*/coliforms	ISO 21528-2:2004	10^2	*RDC n°12 (2001)*
				E. coli O157:H7	and AOAC (1998)	A/25 g	ND
				Salmonella spp.	ISO 16654:2001	A/25 g	*RDC n°12 (2001)*
					ISO 6579:2002		
8	Swab of farmers' hands	3 × 25 cm^2	T3	*E. coli*/coliforms	ISO 21528-2:2004	≤0.7 log cfu/25 cm^2	Jacxsens. et al. (2010)
					and AOAC (1998)	(below detection)	
9	Swab of transport boxes of lettuce	3 × 50 cm^2	T3	*E. coli*/coliforms	ISO 21528-2:2004	≤0.7 log cfu/25 cm^2	Jacxsens. et al. (2010)
					and AOAC (1998)	(below detection)	
10	Irrigation water supply	100 ml	T0	*E. coli*/coliforms	20 TH APHA (1998)	2 × 10^2 cfu/100 ml	*CONAMA. n°357 de 2005*
			T1	Enterococci	20 TH APHA (1998)	A/100 ml	Jacxsens. et al. (2010)
			T2	*E. coli* O157:H7	ISO 16654:2001	A/25 ml	ND
			T3	*Salmonella* spp.	ISO 6579:2002	A/25 ml	ND
11	Irrigation water from tap	100 ml	T0	*E. coli*/coliforms	20 TH APHA (1998)	2 × 10^2 cfu/100 ml	*CONAMA. n°357 de 2005*
			T1	Enterococci	20 TH APHA (1998)	A/100 ml	Jacxsens. et al. (2010)
			T2	*E. coli* O157:H7	ISO 16654:2001	A/25 ml	ND
			T3	*Salmonella* spp.	ISO 6579:2002	A/25 ml	ND
12	Rinse water	100 ml	T3	*E. coli*/coliforms	20 TH APHA (1998)	2 × 10^2 cfu/100 ml	*CONAMA. n°357 de 2005*
				Enterococci	20 TH APHA (1998)	A/100 ml	Jacxsens. et al. (2010)
				E. coli O157:H7	ISO 16654:2001	A/25 ml	ND
				Salmonella spp.	ISO 6579:2002	A/25 ml	ND

[a] A: absent; ND: not defined by official regulation.

using the multiple-tube technique, was applied (Standard Methods for Examination of Water and Wastewater, 1998).

To detect *E. coli* O157:H7, the methodology described in ISO 16654:2001 (ISO, 2001) was used. To confirm presumptive colonies, these were sent to the Brazilian reference Laboratory of *Enterobacteriaceae* at the Bacteriology Department of the Instituto Oswaldo Cruz, Fundação Oswaldo Cruz Foundation (FIOCRUZ).

To analyze *Enterococci* spp., the method described in the manual of methods of microbiological analysis of water (APHA, 1998) was adapted and used. Aliquots of 0.1 ml were spread on M-enterococcus Agar (Himedia, Mumbai, India). The confirmation of the typical colonies was made by Gram coloration and biochemical tests, such as the catalase test, growth on Bile Esculin Agar at 45 °C, and growth at the presence of 6.5% NaCl.

2.3. Self-assessment questionnaire to measure the implementation of good agricultural practices

The self-assessment tools used to interview the farmers consist of a series of indicators. The tools address core activities in the prevention and control of microbiological, mycotoxin, and pesticide residue contamination (e.g., fertilizer program), the context of the farm (e.g., organization and its workforce composition), and the output of its system (e.g., visual complaints). The tools are designed to gain insight into the level of good agricultural practices that are currently applied in the farm (Kirezieva, Jacxsens et al., 2013; Kirezieva, Nanyunja et al., 2013). In this case study, only indicators that were related to potential microbiological contamination or growth were retained (a total of 57 indicators).

The self-assessment questionnaire was organized into four major parts. The first part of the questionnaire consists of a description of the context in which the farm needs to operate; it includes a set of indicators for product and process characteristics, as well as organizational and environmental characteristics. For each indicator, a risk level can be attributed (i.e., low-risk, medium or high-risk level). A low risk indicates that the specific topic of the indicator is not imposing an additional factor for the potential contamination or outgrowth of pathogens to the crop. Meanwhile, a high risk creates additional pressure and challenges for the implementation of the good agricultural practices.

An example of a context indicator for organizational characteristics is "variability in the workforce." In the case of a large turnover of workers, who often do not even speak the language of the country and have poor motivation due to the low attractiveness of the work, a high risk level can be attributed. Because this situation creates difficulties and requires additional efforts for the correct

application of GAP, for example, by translating the procedures, additional training in other languages and different approaches for instructions are necessary (Kirezieva, Jacxsens et al., 2013).

The second and third parts of the questionnaire include a self-assessment of control, assurance activities, and grids with concise descriptions to indicate the levels at which certain good agricultural practices are designed or implemented in practice (Kirezieva, Nanyunja et al., 2013). The levels 1, 2, 3, or 4 represent situations that are nonexistent (1); basic-simple (based on our own or historical knowledge of the farmers) (2); average-common (based on legal requirements or sector guidelines) (3); or advanced-sophisticated (tailored to the specific situation on the farm) (4); respectively for control and assurance activities. An example of an indicator for control activities is the "specificity of fertilizer program"; this is because a site-specific organic fertilizer program with effective composting, supported by appropriate instructions, better prevents pathogen introduction, which will positively contribute to microbiological food safety (level 4). An example of assurance activity is "documentation." It is assumed that a dedicated documentation system, which is adapted to the nature and size of the farm, leads to additional insights in the implementation of good practices and the output of the farm. Therefore, a tailored documentation system leads to a level 4 (Kirezieva, Nanyunja et al., 2013).

A fourth set of indicators was the information about the output of current good agricultural practices at the farm. The four levels could be attributed as: no information of good agricultural practices output was available, low, medium, or high output of the system (Kirezieva, Nanyunja et al., 2013). An example of an indicator for the system output is a "type of visual quality complaints." This is because it is assumed that a low amount of or no complaints of the visual quality of the crop indicates a good performance, when a good complaint registration and evaluation system is in place.

The questionnaire was used during an interview with the individual responsible for the production at the farms. For each indicator, the interviewer in charge had to choose which level was the most representative for the farm's situation. Each interview lasted for approximately two to 3 h and was followed by an on-site visit to confirm the assessment. Mean scores were calculated by using all indicators, respectively, divided by their total amount of indicators.

3. Results

3.1. Microbiological sampling

Table 2 presents the results of samples of seedlings, lettuce, transport boxes, hands of workers, soil, and manure, from Farms 1, 2, or 3, while Table 3 demonstrates the results of irrigation and wash/rinse water samples (CSL 10–12). Overall, a total of 132 samples (44 samples per company) were taken over the three-month period. The manure samples, collected at T_0 on all farms, presented *E. coli* counts ranging from 3.4 to 5.6 $\log_{10}$ cfu/g and coliforms counts from 4.6 to 6.7$\log_{10}$ cfu/g. The presence of *Salmonella* spp. was detected only at Farm 2 (Table 2), and no *E. coli* O157 was detected in the fertilizer/manure samples tested.

The manured soil samples contained *E. coli* counts ranging widely from farm to farm. In all farms, the *E. coli* count started at elevated counts and decreased over time during the sampling period T_0, T_1, T_2, and T_3. For example, at Farm 1, the mean *E. coli* decreased from 4.4 (T_0) to 3.3 (T_1), to 2.3 (T_2) to less than 1.0 log cfu/g (T_3). On the other hand, the counts for coliforms remained barely unaltered over time at all farms. *Salmonella* spp. was detected in Farm 2 at T_0 and in Farm 1 at T_1. *E. coli* 0157 was not identified in any of the manured soil samples (Table 2).

Lettuce seedlings that were collected at T_0 presented *E. coli* counts of less than 1.0 $\log_{10}$ cfu/g, while the coliform counts ranged

Table 2
Microbial results of samples collected according to a risk-based sampling plan at three organic lettuce farms of southern Brazil. Mean and standard deviation are expressed as log cfu g[a] or cfu cm^{2}[b] for *E. coli* and coliforms. For the pathogens *Salmonella* and *E. coli* O157:H7, the results are expressed as presence or absence in 25 g or in 100 cm^2 (boxes) or on hand (workers' hands).

Visit	CSL	Description	Farm 1					Farm 2				Farm 3			
			Number samples	*E. coli*[a]	Coliforms[a]	*Salmonella* spp.[b]	*E. coli* O157:H7	*E. coli*[a]	Coliforms[a]	*Salmonella* spp.	*E. coli* O157:H7	*E. coli*[a]	Coliforms[a]	*Salmonella* spp.[b]	*E. coli* O157:H7
T0	1	Manure[a]	3	4.3 (±0.11)	4.6 (±0.11)	−(0/3)	−(0/3)	5.6 (±0.50)	6.6 (±0.09)	+(1/3)	−(0/3)	3.4 (±0.41)	4.7 (±0.05)	−(0/3)	−(0/3)
T0	2	Manured Soil[a]	3	2.1 (±0.17)	3.4 (±0.24)	−(0/3)	−(0/3)	4.4 (±0.09)	5.5 (±0.46)	+(1/3)	−(0/3)	2.8 (±1.09)	4.2 (±0.08)	−(0/3)	−(0/3)
T0	3	Soil Seedling[a]	1	<1.0	4.3	−(0/1)	−(0/1)	4.5	4.9	−(0/1)	−(0/1)	2.4	3.4	−(0/3)	−(0/3)
T0	4	Seedling[a]	1	<1.0	3.3	−(0/1)	−(0/1)	<1.0	4.6	−(0/1)	−(0/1)	<1.0	4.6	−(0/3)	−(0/3)
T1	5	Manured Soil[a]	3	<1.0	3.9 (±0.34)	+(1/3)	−(0/3)	3.3 (±0.32)	4.3 (±0.56)	−(0/3)	−(0/3)	2.3[b]	2.7 (±0.67)	−(0/3)	−(0/3)
T1	6	Lettuce[a]	3	<1.0	3.7 (±0.68)	−(0/3)	−(0/3)	3.4 (±0.31)	3.7 (±0.59)	+(1/3)	−(0/3)	2.7(±0.39)[a]	3.3 (±0.16)	−(0/3)	−(0/3)
T2	5	Manured Soil[a]	3	<1.0	3.9 (±0.34)	−(0/3)	−(0/3)	2.3[b]	3.6 (±0.21)	−(0/3)	−(0/3)	<1.0	3.5 (±0.12)	−(0/3)	−(0/3)
T2	6	Lettuce[a]	3	2.4[b]	4.0 (±1.34)	−(0/3)	−(0/3)	<1.0	3.8 (±1.42)	−(0/3)	−(0/3)	<1.0	2.5 (±0.63)	−(0/3)	−(0/3)
T3	5	Manured Soil[a]	3	2.3[b]	3.3 (±0.31)	−(0/3)	−(0/3)	<1.0	4.4 (±0.30)	−(0/3)	−(0/3)	2.3[b]	3.2 (±0.23)	−(0/3)	−(0/3)
T3	6	Lettuce[a]	3	3.6 (±0.21)	3.9 (±0.95)	−(0/3)	−(0/3)	<1.0	3.8 (±0.06)	−(0/3)	−(0/3)	3.1[b]	3.2 (±0.82)	−(0/3)	−(0/3)
T3	7	Lettuce (final product)	3	<1.0	3.0 (±0.31)	−(0/3)	−(0/3)	<1.0	3.8 (±0.06)	−(0/3)	−(0/3)	<1.0	2.8 (±0.48)	−(0/3)	−(0/3)
T3	8	Workers' Hands[b]	3	<1.0	1.8 (0.58)[a]	−(0/3)	−(0/3)	<1.0	3.3 (±0.26)	−(0/3)	−(0/3)	1.9[b]	2.5 (±0.94)	−(0/3)	−(0/3)
T3	9	Boxes[b]	3	<1.0	2.1 (±0.98)	−(0/3)	−(0/3)	<1.00	3.5 (±0.41)	−(0/3)	−(0/3)	<1.0	2.5 (±0.94)	−(0/3)	−(0/3)
		Total	35			1/35	0/35			3/35	0/35			0/35	0/35

All of the values are presented as the mean and standard deviation except for seedling and soil seedlings.
To: Start of the planting. T1: three weeks before harvest. T2: two weeks before harvest. T3: harvest.
[a] Result of one sample was below the detection limit (<1.00 log).
[b] Results of two samples were below the detection limit (<1.00 log).

Table 3
Results of irrigation water and rinse water collected at three organic lettuce farms in Southern Brazil. Mean and standard deviation are expressed as MPN/100 ml or *E. coli* and coliforms. For the pathogens *Salmonella* and *E. coli* O157:H7, the results are expressed as presence or absence in 25 ml. For *Enterococcus* spp., the results are expressed as presence or absence in 100 ml.

Visit	CSL	Description	Samples	Farm 1					Farm 2					Farm 3				
				E. coli MPN/100 ml	Coliforms MPN/100 ml	*Enterococcus* 100 ml	*Salmonella* spp. 25 ml	O157:H7 25 ml	*E. coli* MPN/100 ml	Coliforms MPN/100 ml	*Enterococcus* 100 ml	*Salmonella* spp. 25 ml	O157:H7 25 ml	*E. coli* MPN/100 ml	Coliforms MPN/100 ml	*Enterococcus* 100 ml	*Salmonella* spp. 25 ml	O157:H7 25 ml
TO	10	Irrigation water source	1	>23	>23	Absence	Absence	Absence	16.10	16.10	Absence	Absence	Absence	23	>23	Absence	Absence	Absence
TO	11	Irrigation water tap	1	>23	>23	Absence	Absence	Absence	>23	>23	Absence	Absence	Absence	16.1	23	Absence	Absence	Absence
T1	10	Irrigation water source	1	12.0	23	Absence	Absence	Absence	3.6	>23	Absence	Absence	Absence	>23	>23	Absence	Absence	Absence
T1	11	Irrigation water tap	1	12.0	23	Absence	Absence	Absence	1.1	>23	Absence	Absence	Absence	1.1	16.1	Absence	Absence	Absence
T2	10	Irrigation water source	1	5.1	>23	Absence	Absence	Absence	6.9	9.20	Absence	Absence	Absence	9.2	12	Absence	Absence	Absence
T2	11	Irrigation water tap	1	3.6	>23	Absence	Absence	Absence	6.9	12	Absence	Absence	Absence	9.2	16.1	Absence	Absence	Absence
T3	10	Irrigation water source	1	1.1	23	Absence	Absence	Absence	1.1	23	Absence	Presence	Absence	23	23	Absence	Absence	Absence
T3	11	Irrigation water tap	1	6.9	23	Presence	Absence	Absence	>23	>23	Absence	Absence	Presence	>23	>23	Absence	Absence	Absence
T3	12	Rinse water total	1 9	1.1	5.1	Absence	Absence	Absence	>23	>23	Absence	Absence	Absence	>23	>23	Absence	Absence	Presence

from 3.3 to 4.6 $\log_{10}$ cfu/g. *Salmonella* spp. and *E. coli* O157 were not detected (Table 2).

The lettuce samples presented *E. coli* counts ranging from less than 1.0 to 3.6 $\log_{10}$ cfu/g. No trend over time was observed, and the contamination variability was detected between farms and the time of sampling. For example, at Farm 1, the average counts were less than 1.0 $\log_{10}$ cfu/g at T1 and 2.6 $\log_{10}$ cfu/g at T3. At Farm 2, the counts at T_1 started with 3.4 $\log_{10}$ cfu/g and decreased to less than 1.0 $\log_{10}$ cfu/g at T_3. At Farm 3, the average counts at T1 were 2.7 $\log_{10}$ cfu/g and decreased to 1.0 $\log_{10}$ cfu/g at T_2 and increased again to 3.1 $\log_{10}$ cfu/g at T3. In spite of this, all of the lettuce samples from the three producers presented low counts (less than 1.0 log cfu/g) after the final wash with water. This indicates that washing was able to reduce the microbial load. *Salmonella* spp. was isolated from a lettuce sample only at Farm 2 at T_1 (Table 2). Moreover, *E. coli* O157 was not detected in any of the samples of the producers at any of the sampling instances.

Irrigation water samples collected in both pond and taps (sprinklers) presented contamination by *E. coli* (from 1.1 to >23 MPN/ml) and coliforms (from 12 to >23 MPN/ml) at all times. The counts were similar throughout the sampling period (Table 3). Only at Farm 1, there was a tendency of decreasing *E. coli* counts. *Enterococcus* spp. was determined in sprinkler water at T_3 (Table 3) and *Salmonella* spp. Furthermore, *E. coli* O157 was found on Farm 2 at T_3 in irrigation water and irrigation tap water, respectively (Table 3).

All of the rinse water samples collected after washing the lettuce presented contamination with *E. coli* and total coliforms (Table 3). At Farm 1, which used potable water to wash lettuce, the counts were low (1.1 and 5.1 MPN/ml of *E. coli* and total coliforms, respectively). At Farms 2 and 3, the counts were beyond 23 MPN/ml. Moreover, at these farms, the water to prepare lettuce plants for the market was obtained from a well and from a pond, respectively.

Contamination by *Salmonella* spp. was not detected in any sample of rinse water collected at the three farms. Nonetheless, at Farm 3, rinse water contamination by *E. coli* O157 was identified (Table 3).

The box samples collected at all three farms showed relatively low counts of *E. coli* (less than 1.0 $\log_{10}$ cfu/cm^2), whilst total coliforms ranged from 2.1 to 3.5 $\log_{10}$ cfu/cm^2 (Table 2).

The samples collected from the workers' hands at the moment of the lettuce harvest (T_3) indicated the presence of *E. coli* and total coliforms at all three farms. The *E. coli* counts maintained a pattern of contamination, ranging from less than 1.0up to 1.9 $\log_{10}$ cfu/hand, while the total coliform counts ranged from 1.8 to 3.3 $\log_{10}$ cfu/hand (Table 2).

3.2. Self-assessment questionnaire of the implementation of good agricultural practices (GAP)

The details of the results of the self-assessment questionnaire are shown in Table 4. All three surveyed organic lettuce farms operate in a moderate to high level of risk, regarding product and process characteristics, as the indicators related to product and process characteristics scored for the calculated mean 3.0 (Farms 1 and 3) or 2.8 (Farm 2). The indicators of organization and chain characteristics obtained a calculated mean of 2.3 (Farm 1) and 2.4 (Farms 2 and 3) (moderate to high level of risk). Two indicators (i.e., "technological farm team" and "variability in the workforce") were different and more dependent on the farm's own operation. Farm 1 and Farm 2 included the assistance of a professional agronomist, who increased technological capacity, while Farm 3 depended on the historical knowledge of the farmers. Farm 2 showed a high employee turnover that was not observed at the other farms.

The indicated levels of the control and assurance activities of the farmers are shown in detail on Table 4 (part II and III). The calculated mean score of the design for control activities was 2.0. This indicates

Table 4
Levels attributed to the indicators representing context factors, core control, and core assurance activities in good agricultural practices and the output based on self-assessment questionnaires from three lettuce production farms in southern Brazil.

Indicators	Assumption linked to indicator (based on Kirezieva, Jacxsens et al., 2013, Kirezieva, Nanyunja et al., 2013)	Farm 1	Farm 2	Farm 3
PART I. Context factors[a]				
Product and process characteristics				
Risk of raw materials (microbial)	Initial materials that are more prone to microbial contamination, growth, and survival due to their natural characteristics and/or cultivation practices increase chances of lower food safety performances and put higher requirements on GAPs, resulting in lettuce seedlings that can be associated with microbiological contaminations.	3	3	3
Risk of final product (microbial)	Products that are susceptible to pathogen or fungal growth due to their surface properties increase chances of lower food safety performance and put higher requirements on GAPS, resulting in lettuce crops that are associated with microbiological hygiene or pathogen prevalence.	3	3	3
Production system	Production/cultivation systems that are more susceptible to microbial contamination due to their contact with the soils and the environments increase chances of lower food safety performances and put higher demands on GAPs, resulting in open field production.	3	3	3
Climate conditions	Climate conditions of production environments that favor growth of microorganisms and/or occurrence of pests, increase chances of lower food safety performances and put higher demands on GAPs. This region of Brazil is characterized by a warm and humid climate.	3	3	3
Water supply	Water supplies for direct contact with products that have high likelihoods of contamination by microorganisms and/or chemicals (i.e., uncontrolled surface water, water from ponds) increase the chances of lower food safety performances and put higher demands on GAPs.	3	2	3
Mean product and process		*3.0*	*2.8*	*3.0*
Organization and chain				
Presence of technological staff	Companies with limited or no specific internal and external expertise in food safety are less able to make underpinned decisions, which negatively affects hygiene and food safety and puts demands on GAPs, resulting in farms having low technological knowledge (e.g., no external technical support or activities done that are based on empirical knowledge).	1	1	3
Variability in workforce composition	Variability in workforce composition due to part-time workers and high personnel turnover may result in loss of company-specific experience, which can increase chances of poor execution of safety tasks, which negatively influences hygiene and food safety, putting demands on GAPs (e.g., by requiring robust procedures or more operator control from experienced farmers or supervisors).	1	3	1
Sufficiency of operator competences	Recruited operators with inadequate education levels, lacks of experience, and restricted training supports increase chances of poor execution safety tasks, which negatively affects hygiene and food safety, putting demands on GAPs (e.g., by requiring robust procedures for specific workers, having different languages, or requiring more operator control).	2	2	2
Extent of management commitment	Lack of management commitment on food safety control shifts priorities of employees/operators to other issues, which increases chances of poor operation (e.g., by not following procedures adequately), and puts higher demands on GAPs (e.g., by requiring advanced control and assurance activities).	3	3	3
Degree of employee involvement	Lack of employee involvement will result in less committed and motivated operators, which favors inappropriate operation and puts higher demands on GAPs (e.g., by requiring more instructions, training, and operator control).	2	2	2
Level of formalization	Absence of establishment of activities in formal procedures and lack of formalized meetings increase chances of unexpected decision-making behavior with safety tasks and put higher demands on GAPs (e.g., by requiring advanced control activities).	3	3	3
Sufficiency supporting information systems	Lack of appropriate information systems affects availability of accurate information, which may favor inappropriate operation due to lack of (correct) info of safety tasks and put higher demands on GAPs by requiring advanced control and assurance activities (e.g., increased efforts in obtaining appropriate information at the right time and place).	3	3	3
Severity of stakeholders requirements	**Strict** and **differing** requirements on your GAPs set by stakeholders (government, branch organizations, customers, retailers, etc.) puts higher demands on GAPs by requiring advanced control and assurance activities.	1	1	1
Extent of power in supplier relationships	Lack of power in the supplier relationship means less influence of a company on their suppliers, which may result in more unpredictable safety levels of incoming materials, putting higher demands on GAPs (e.g., by requiring advanced incoming material control and supplier control).	2	2	2
Food safety information exchange	Companies that lack systematic information sharing with their suppliers have to deal with less predictable safety levels, which puts demands on GAPs (e.g., requiring advanced control measures).	3	3	3
Logistic facilities	Lack of adequate and strictly controlled environmental conditions of logistic facilities increases chances of undesired growths of microorganisms or contamination, which puts demands on GAPs (e.g., by requiring advanced monitoring, validation, verification).	3	3	3
Inspections of food safety authorities	Lack of systematic procedure-driven inspections and adequate feedback by acknowledged food safety authorities leads to less reliable feedback information about the GAPs performances to companies, putting demands on GAPs by requiring more advanced assurance activities (e.g., verification and validation).	3	3	3
Supply source of initial materials	Companies purchasing initial materials from suppliers with variable food regulations have an increasing chance of unknown hazards and unexpected contamination, putting demands on GAPs by requiring more advanced control and assurance activities (e.g., incoming materials control, verification).	3	3	3
Specific external support	Lack of specific product or production system external support will increase the chances of inadequate safety decisions, which may lead to food safety problems, putting more requirements on GAPs (e.g., requiring more testing of actual situations, advanced validation).	3	3	3
Specific legislation	Lack of a well-established and detailed national food policy with specifically defined legislative acts on food safety will increase chances for inadequate safety decisions, which puts demands on GAPs (e.g., by requiring advanced control measures).	1	1	1
Mean organization and chain		2.3	2.4	2.4

Table 4 (*continued*)

Indicators	Assumption linked to indicator (based on Kirezieva, Jacxsens et al., 2013, Kirezieva, Nanyunja et al., 2013)	Farm 1	Farm 2	Farm 3
PART II. Control activities[b]				
Design of control activities[b]				
Hygienic design of equipment and facilities	Advanced hygienic designs of critical equipment and facilities decreases chances of (cross) contamination and enables effective cleaning, which will positively contribute to food safety.	1	1	1
Maintenance and calibration program	Structural and tailored programs for maintenance with specific instructions about frequency and tasks will cause fewer unexpected safety problems due to unreliable equipment, which will positively contribute to food safety.	1	1	1
Storage facilities	More adequate storage facilities better maintain strict temperature and/or atmospheric conditions to prevent growth of microorganisms, which will positively contribute to food safety.	2	2	2
Sanitation program(s)	Specific, full-step, and tailored sanitation programs with appropriate cleaning agents supported by appropriate instructions better prevent contamination, which will positively contribute to food safety.	1	1	1
Personal hygiene requirements	Higher and more specific personal hygiene requirements and specific instructions reduce chances of contamination, which will positively contribute to food safety.	2	2	2
Incoming material control	Systematic and adequate incoming material control will prevent (high and variable initial) acceptance of contaminated incoming materials, which will reduce chances of (cross) contamination of the production process, positively contributing to food safety.	2	2	2
Packaging equipment	Capable packaging equipment results in less unpredictable process variation and better compliance to standards, which will positively contribute to food safety.	1	1	1
Supplier control	More systematic supplier selection and evaluation will lead to more predictable safety levels of incoming materials, which will positively contribute to food safety.	3	3	3
Organic fertilizer program	Site-specific organic fertilizer programs with capable composting supported by appropriate instructions better prevent cross-contamination and positively contribute to food safety.	2	2	2
Water control	Systematic monitoring and adequate water treatment will prevent (high and variable initial) contamination, which will positively contribute to food safety.	1	1	1
Irrigation method	Irrigation methods that are specifically aimed at avoiding direct contact with edible parts of produce will better prevent **microbiological** contamination, which will positively contribute to food safety.	2	2	2
Partial physical intervention (washing, rinsing)	Capable partial physical intervention enables less unpredictable process variation and better compliance to standards, which will positively contribute to food safety.	2	3	3
Analytical methods to assess pathogens	Sensitive, specific, repeatable, reproducible, and rapid methods to assess pathogens will result in more adequate determinations of pathogens, which will positively contribute to food safety.	3	1	1
Sampling plan for microbial assessment	A statistical underpinned and tailored sampling plan increases reliability of information on actual product/process status, which will positively contribute to food safety.	1	1	1
Corrective actions	A complete and differentiated description of corrective actions linking severity of deviations to type of corrective actions will positively contribute to food safety.	1	1	1
Mean control activities design		*2*	*2*	*2*
Control activities operation[b]				
Actual availability of procedures	Accurate and understandable procedures at the right places will better direct peoples' decision-making behaviors in control, which will positively contribute to food safety.	1	1	1
The actuality of compliance to procedures	Complete (all steps followed) and accurate (in the right way) compliance to procedures will result in more appropriate decision-making behavior in control, which will positively contribute to food safety.	2	2	2
Actual hygienic performance of equipment and facilities	Stable hygienic performance of equipment and facilities will result in less (cross) contamination, which will positively contribute to food safety.	1	1	1
Actual storage/cooling capacity	Stable performances of storage/cooling facilities will result in constant parameters with fewer variations, which will better prevent growth of microorganisms and will positively contribute to food safety.	1	1	1
Actual process capability of partial physical intervention	Stable intervention processes with minor differences between different lines/batches and well noticeable capability performances will result in more in-spec products (within specifications), which will positively contribute to food safety.	1	1	1
Actual process capability of packaging	Stable packaging with minor differences between different production lines/batches and well noticeable capability performances will result in more in-spec products (within specifications), which will positively contribute to food safety.	1	1	1
Actual performance of analytical equipment	Stable measuring equipment that is reliable under different product/process conditions provide more reliable information on product and process status, which will positively contribute to food safety.	1	1	1
Mean control activities operation		*1.1*	*1.1*	*1.1*
PART III. Assurance activities[b]				
Translation of stakeholder requirements into own HSMS requirements	Systematic and precise translation of stakeholder requirements will result in suitable requirements on the GAPs, which will contribute to assurance of product safety.	1	1	1
The systematic use of feedback information to modify HSMS	Systematic use of valid feedback information from control systems will result in appropriate system modifications, which will contribute to assurance of product safety.	1	1	1
Validation of preventive measures	A scientific, evidence-based, systematic, and independent validation of effectiveness of selected preventive measure will result in effective GAPs, which will positively contribute to assurance of product safety.	1	1	1
Validation of intervention processes	A scientific, evidence-based, systematic, and independent validation of effectiveness of selected intervention processes will result in more effective GAPs, which will positively contribute to assurance of product safety.	1	1	1
Verification of people-related performance	A more specific, systematic, and independent verification of procedure characteristics and compliances will result in more reliable GAPs, which will positively contribute to assurance of product safety.	1	1	1
		1	1	1

(*continued on next page*)

Table 4 (*continued*)

Indicators	Assumption linked to indicator (based on Kirezieva, Jacxsens et al., 2013, Kirezieva, Nanyunja et al., 2013)	Farm 1	Farm 2	Farm 3
Verification of equipment and methods related performance	A more specific, systematic, and independent verification of equipment and method performances will result in more reliable GAPs, which positively contributes to the assurance of product safety.			
Documentation system	An integrated, up-to-date, and accessible documentation system will improve information (experience, scientific knowledge, legislative requirements) for GAPs, which will support validation and verification activities, positively contributing to the assurance of product safety.	1	1	1
Record-keeping system	A structured, integrated, and accessible record-keeping system will support validation and verification activities, which will positively contribute to assurance of product safety.	1	1	1
Mean assurance activities		*1.0*	*1.0*	*1.0*
PART IV. System output[c]				
Evaluation of good agricultural practices	A certification audit by a third party or an inspection by the national food safety agency gives an external and independent evaluation of the current GAPs.	1	1	1
Seriousness of remarks	A positive evaluation (without serious remarks) of the GAPs by a national food safety agency and/or accredited third party indicates a good safety performance (i.e., that all requirements of the stakeholders are met).	1	1	1
Hygiene-related and microbiological food safety	The presence of a good functioning system for **complaint** registration and evaluation of complaints is an important aspect in GAPs. Low number of or no complaints regarding **hygiene and microbiological food safety of final products** indicates a good performance of food safety.	1	1	1
Typify the visual quality complaints	The presence of a good functioning system for complaint registration and evaluation of complaints is an important aspect in GAPs. Low number or no complaints of visual quality indicates a good performance of food safety.	1	1	1
Product sampling for microbiological performance	Structured sampling and different types of samples give a more comprehensive and accurate indication of the actual microbiological performance of GAPs.	1	1	1
Judgment criteria for microbiological results	Using more criteria to critically interpret obtained results of microbiological analyses gives a more accurate indication of the microbiological performance of the GAPs.	1	1	1
Non-conformities	The presence of a good system for **non-conformities** registration and evaluation gives a good indication of the performance of GAPs. Low number of or no non-conformities indicates a good food safety performance.	1	1	1
Mean system output		*1.0*	*1.0*	*1.0*

[a] For context (part I), product and process characteristics as well as organization and chain characteristics are assessed based on three risk levels: level 1 represents low risk level; level 2, medium; and level 3, high risk level of additional contamination or growth on crops.

[b] For control (split in the designs of control activities and actual operation or implementation of control activities) (part II) and assurance activities (part III) in good agricultural practices, four levels can be selected: level 1 is non-existing, not implemented; level 2, activity done at basic level based on known insights and historical information; level 3, activity set up and implemented based on sector information or guidelines; and level 4, the activity is adapted and tailored to the specific situation on the farm.

[c] For system output indicators (part IV) also, four levels can be attributed: level 1, not done, no information is available; level 2, limited information is available, ad hoc sampling is performed; level 3, more systematic information is available; and level 4, systematic information is available and good results are obtained, e.g., no complaints, no problems related to visual quality, and no major remarks during inspections or audits.

that these activities are conducted on a basic level, using historical and common knowledge. However, no sector information or information from suppliers was applied (level 3) or tailored to the farm's own situation (level 4). The results of the design for control activities in the Good Agricultural Practices were very similar for the three farms. However, Farm 1 differs from Farms 2 and 3 by the partial physical intervention (the washing step) that was conducted at a basic level at Farm 1. To the contrary, at Farms 2 and 3, partial physical intervention was executed, based on sector information (level 3). The indicator "analytical methods applied for microbiological analyses of pathogens" was at level 3 for Farm 1, indicating that the farm was working with accredited laboratories for the completion of microbiological analyses; the other farms did not analyze microbiological indicators or pathogens (level 1). For the indicators related to the hygienic design of the equipment, maintenance program, sanitation program, packaging equipment, water control, and corrective actions, all of the farms were operating at level 1, indicating not conducted or not done at all three farms.

The operation of the control activities and assurance activities in farms were very low (calculated mean of 1.1 and 1, respectively), indicating that these activities were not implemented or applied in practice (Table 4). Also, the general output of the current implemented good agricultural practices in the organic lettuce farms was also low (mean 1 for the three farms in part IV system output, Table 4). The reason for this is because no information of the system output was available; no inspection or audit was performed, no samples (both microbiological and chemical) were taken, so no actual evaluation of their system output could be performed.

4. Discussion

All three farms received technical support, related to organic production practices, that was provided by regulatory bodies and organic farm associations. The focus was mainly on the control of chemical hazards, such as pesticide residue, as could be derived from the interviews with the farmers. The workers were very compliant, responsive to changes, and concerned with possible quality improvements. However, the self-assessment questionnaire demonstrated that all farms operated in a high microbial risk context with respect to product and process characteristics (Table 4 – part I). This result could be expected because all included farms are conducting the same type of production process (cultivation of lettuce in open fields), in the same region and climate conditions. Also, all of the work is performed according to organic guidelines. Corroborating these findings, *E. coli* and foodborne pathogens were found among the farms over time, according to the sampling plan (Table 2). Several studies have demonstrated that leafy greens may frequently present contamination by fecal related microorganisms and/or pathogens, due to their natural characteristics and the contact with soil, irrigation water, and animal intrusion (Fischer-Arndt, Neuhoff, Tamm, & Köpke, 2010; James, 2006; Levantesi et al., 2012; Millner, 2003; Moyne et al., 2011; Oliveira, Ritter et al., 2012; Oliveira, Viñas et al., 2012).

Seedlings also may be contaminated, especially when they are not treated with chemicals or have not undergone heat treatments before use, as in the case of the investigated organic farms. However, microbiological analyses demonstrated a very low contamination in

seedlings (Table 2). This suggests that initial contamination was not an important factor in contributing to final lettuce contamination. Based on the microbiological results presented in Table 2, during the growth of the lettuce, the lettuce did become contaminated; this is probably due to several other risk factors, such as the use of insufficiently composted manure or an uncontrolled source and quality of irrigation water.

Even though the self-assessment tool indicated a high risk of the final products' microbiological contamination (Table 4 – part I), all of the samples of washed lettuce (final products) collected from the three farms proved to be in accordance with a Brazilian regulation that established 10^2 cfu/g as the maximum acceptable count of *E. coli* in lettuce (Table 1). Different results were found by the study of Arbos, Freitas, Stertz, and Carvalho (2010) and Santana et al. (2006), in which samples of different lettuce crops had *E. coli* counts above those permitted by the Brazilian regulation. The presence of *E. coli* in vegetables may indicate insufficient awareness of microbial hazards during farming, inadequate sanitary conditions, and an increased probability of contamination by pathogenic bacteria associated with several foodborne illnesses (Neto et al., 2012; Soriano, Rico, Moltó, & Mañes, 2000). The presence of *E. coli* O157 was not detected in the lettuce plant samples throughout cultivation. However, the amount of samples taken in the present study was restricted to pick pathogens, if the prevalence is low (0.1–1%). International references indicated the presence of *E. coli* O157 on leafy greens such as lettuce and spinach (Ackers et al., 1998; FDA, 2007; Oliveira, Ritter et al., 2012; Oliveira, Souza, Bergamini, & Martinis, 2011; Oliveira et al., 2010; Oliveira, Viñas et al., 2012; Santana et al., 2006).

The water supply was considered a medium (Farm 2 used ground water) or high risk (Farms 1 and 3 used pond water) because of the nature of the water source applied for irrigation (Table 4 – part I). The ground water can be contaminated with different kinds of micro-organisms, such as *E. coli, Salmonella* spp. and *Campylobacter* (Fong et al., 2007; Richardson, Nichols, Lane, Lake, & Hunter, 2009). However, the ground water is generally accepted to be of better quality because the water is protected from contamination more than surface water (Richardson et al., 2009). Irrigation water samples, collected in both the water sources and the sprinklers of the surveyed farms, indicated contamination by *E. coli* at all times (Table 3). All of the farmers, except Producer 2, used pond water for irrigation with sprinklers. Meanwhile, Producer 2 used ground water pumped up from a dug well for drip irrigation. The microbiological quality of water was not verified by farmers before use, and the sources were exposed to field contamination, justifying the fecal contamination observed.

The Brazilian regulation (CONAMA resolution 357 of 2005, Table 1) established a limit for *E. coli* of 2×10^2 cfu/100 ml for the irrigation water of vegetables. Based on this limit, in the present study, several water samples were in accordance with the regulation; nevertheless, attention should be given to the frequent presence of *E. coli* indicating fecal contamination. Furthermore, the variation of the counts among the sampling periods also indicates that this limit may be surpassed over time. The irrigation water in the present study showed contamination by *E. coli* O157 in Farm 2, in which the lettuces were ready for harvest, indicating a serious risk of contaminating the final product. Foodborne outbreaks with leafy vegetables, contaminated by water, have been reported by several studies worldwide (Beuchat, 1996; Delaquis et al., 2007; Itohan et al., 2011). Pathogenic bacteria, such as *E. coli* O157, are frequently associated with outbreaks; the outbreaks result from inadequate treatment of the water used for irrigation and the washing of fruits and vegetables (Beraldo & Filho, 2011; Levantesi et al., 2012; Moyne et al., 2011). It is important to note that the *E. coli* O157 was found in the irrigation water of Farm 2 and in the rinse water of Farm 3 after a flood. This suggests that such events could be important sources of contamination. Therefore, specific preventive and control measures should be planned in order to avoid lettuce final product contamination during floods. It is a good idea to set the plantation on elevated areas in which floods cannot affect its microbial quality, avoiding contact with animals and their feces with the water sources. In addition, it is important to collect water from local areas in which natural water is flowing, or from non-contaminated wells. These techniques can be considered as examples of preventive measures, while the discard of plantations affected by floods can be implemented as a control measure to avoid public health problems (Kirezieva, Jacxsens et al., 2013; Liu, Hofstra, & Franz, 2013).

In terms of organization characteristics (Table 4 – part I), the situation was different for the involved farms. The technological staff and workforce variability demonstrate that the technological staff was trained in food safety (Farms 1 and 2) or a stable workforce was in place (Farms 1 and 3). Only in Farm 1 were both cases applicable. Studies showed that a trained technological staff and stable workforce help create a capacity in a company to be able to anticipate food safety questions and problems (Kirezieva, Nanyunja et al., 2013; Luning et al., 2011). Furthermore, a trained and stable workforce composition can create less pressure for the implementation of GAPs because people know the responsibilities of their jobs (Kirezieva, Jacxsens et al., 2013). More organization characteristics of the interviewed farms illustrate that the level of formalization and information system was at a high risk (level 3); this is because no formal method of keeping documents and registrations were implemented. It has been demonstrated that keeping records is indeed very rarely elaborated at a farm level (Jevsnik, Hlebec, & Raspor, 2008; Nieto-Montenegro, Brown, & LaBorde, 2008). Also, the commitment of management and employees toward microbiological food safety was low at all three farms.

This situation is typical for a family based farm, where a low degree of formalization or technological capacity is present (Luning et al., 2011; Powell, Jacob, & Chapman, 2011). Chain characteristics were very similar over all three farms and demonstrated another typical situation for smaller family farms, i.e., logistic facilities were not present and discussions or exchanges of information with suppliers or customers did not occur. Stakeholders' requirements were low for the involved farms, and only the legal requirements to adhere to good agricultural and organic farming practices were regarded by the farms. Governmental enforcement by inspections can lead to better compliance with good practices (Jaffee & Masakure, 2005; Kirezieva, Nanyunja et al., 2013), and external support from sector associations may help. However, in the cases of the farms studied, the external supports seemed not to guide the farmers toward potential microbiological hazards. No direct link could be made between the organization and chain characteristics as for the microbiological results. However, in the work of Kirezieva, Nanyunja et al. (2013) it is assumed that a high risk situation leads to a more vulnerable microbiological outcome for a company.

To design control measures for good practices, a basic level was obtained of the fifteen indicators (mean of design of control activities 2, Table 4 – part II), which indicates that good practices are set up according to the knowledge of the farmers and historical insights (Kirezieva, Jacxsens et al., 2013). However, in actual operation these practices still are lacking in several areas (mean of operation of control activities was only 1.1, Table 4). A similar situation was found throughout the three farms concerning the set up and implementation of good agricultural practices (Table 4). Often there was a discrepancy between knowledge of principles and actual implementation or monitoring of good practices. The daily follow-up and implementation of these practices demands continuous efforts by

the farmers (Michaels & Todd, 2005; Soon & Baines, 2012). Moreover, it has been demonstrated not only with farmers, but also in food industries that preventive measures or interventions are often difficult to conform to continuously throughout production (Oses et al., 2012; Sampers et al., 2010). A direct link between the outcome of self-assessment and the obtained microbiological results can be made and is further discussed below.

Manure samples from the three farms showed high contamination of *E. coli* and coliforms, suggesting that composting times were not appropriate. Corroborating this result, the indicator "organic manure program" revealed that the farms developed organic fertilizer programs based on common farm knowledge, and the efficiency of the composting process to decrease microbial load, fecal indicator, or pathogenic bacteria was not known or tested (indicator on level 2 for the three farms, Table 4). Moreover, storage, frequency of application, and methods of application of manure were derived from the producers' own experiences. These findings strongly suggest that one of the possible control/assurance activities at farms should be the implementation of well-controlled fertilizer programs, focusing mainly on the control of composting times as a preventive measure (Harris et al., 2013). Studies have indicated that the time of compost and temperature of manure may affect microorganisms like *E. coli*, *E. coli* O157:H7, and Salmonella (Fischer-Arndt et al., 2010; James, 2006; MAFF, 2000; Millner, 2003; Oliveira, Ritter et al., 2012; Oliveira, Viñas et al., 2012). Although the presence of *E. coli* O157:H7 was not detected in any of the analyzed fertilizer samples, the presence of this pathogen has been identified in similar studies, suggesting the need for better control within the agricultural production (Islam, Doyle, Phatak, Millner, & Jiang, 2005; MAFF, 2000; Oliveira, Ritter et al., 2012; Oliveira, Viñas et al., 2012).

The presence of *Salmonella* spp. and a high count of *E. coli* were detected in the manure from Farm 2. This fertilizer was prepared with horse feces and was composted for a period of 90 days, which may not have been enough time for the reduction of pathogens. The contamination indicates a high potential for microbiological risk. As shown in the studies of Johannessen (2005) and MAFF (2000), the presence of *Salmonella* spp. indicates a serious problem of improperly composted fertilizer because *Salmonella* spp. is a pathogenic microorganism and food products like lettuce are eaten raw. These studies include that contamination of fertilizer may contaminate irrigation water and soil, and that contamination could be a source that spreads to lettuce plants. Both from the microbiological viewpoint and by the self-assessment tool, it is clear that managing manure, compost, and time before applying fertilizer to the field/produce needs further attention and was not under control at the farms.

Another indicator that can be linked to the microbiological results is "partial physical intervention," or the washing step that is conducted to reduce the microbial load. There are studies that point to the washing of lettuce plants as an effective measure to reduce up to one log the microbiological contamination (Bobco et al., 2011; Oliveira, Ritter et al., 2012; Oliveira, Viñas et al., 2012). However, if the washing process is done with reutilized water or with standstill water, then an increase in the microbiological contamination of the final product may occur (Antunes, 2009). According to the self-assessment questionnaire, the washing process was conducted at Farms 2 and 3 according to sector guidelines, while at Farm 1 the washing process was only based on common and historical knowledge. Based on these findings, control of the quality of water seems to be one of the most important interventions to be implemented on farms. The effect of washing on the *E. coli* count of the lettuce can be derived by comparing CSL 6 and 7 as in Table 2.

For Farms 1 and 3, a two log reduction of contamination can be seen after washing, while for Farm 2 no effect of washing could be seen due to the already low contamination of the lettuce before washing (<1 log cfu/g). However, none of the farms had a water control program, as evident from the self-assessment questionnaire and indicator "water control" (on level 1, Table 4), making obvious the high risk level if additional control measures are not implemented. The water used in rural areas for washing lettuce intends to remove debris and to reduce contamination of the vegetables. Yet if the water is contaminated, the contamination of lettuce plants will probably increase as well. In the present study, the rinse water from Farm 3 showed contamination by *E. coli* O157:H7; nevertheless, the lettuce plants washed in the water did not show contamination. It is possible that the contamination of the lettuce by *E. coli* 0157:H7 was reduced by washing and below the detection limit of the method. Based on the fact that end users often only wash vegetables before consumption and that home washing may not be sufficient to eliminate pathogens, the microbial quality of the rinse waters at production sites must be considered critical for the safety of lettuce plants (James, 2006; Moyne et al., 2011; Oliveira, Ritter et al., 2012; Oliveira, Viñas et al., 2012).

In this context, it may be necessary to consider implementing preventive measures on farms and emphasizing the sanitizing of lettuces in the homes of consumers or food services before consumption in order to increase safety. According to the food services regulation Portaria Estadual of Rio Grande do Sul no. 78 of 2009 (Rio Grande do Sul, 2009), vegetables prepared in food services should be washed and disinfected using 100–250 ppm solution of free chlorine for 15 min and then be rinsed with potable water.

If the self-assessment questionnaire is further followed, we can derive that assurance activities were not in place so far in this case study (part III, Table 4, mean level of assurance activities is 1). Working out assurance activities can be seen as a next step in evolving good practices toward a food safety management system (Jacxsens, Devlieghere, & Uyttendaele, 2009a; Jacxsens, Kussage et al., 2009; Kirezieva, Jacxsens et al., 2013a). The objective of assurance activities is to provide transparency and confidence to the stakeholders (e.g., customers, government) that good practices can overcome the related hazards (Taylor, Kastner, & Renter, 2010; Yudin, 2011). As the involved farms do not yet have good agricultural practices in place, it is logical that assurance activities are not yet elaborated upon. Other studies reveal that assurance activities are lacking at farm level (Okello & Swinton, 2007) and even in the food industry (Oses et al., 2012; Sampers, Toyofuku, Luning, Uyttendaele, & Jacxsens, 2012).

Also, in the information about system output (part IV of the self-assessment questionnaire, mean level 1), it is clear that the involved farmers did not gather information related to whether they perform well as none of the indicators were yet in place. Collecting information on the produced product (e.g., by microbiological status, visual quality, or pesticide residues) or the current goodness of agricultural practices (e.g., by follow-up actions to the non-compliances noticed at the farms, getting input from inspections, or audits) can give insight to the farmer about what aspects of production could be improved (Jacxsens et al., 2010).

5. Conclusions

The sampling of products and environments in combination with the self-assessment questionnaire allowed us some insights into the microbiological safety and hygiene statuses of the production chains of organic lettuce with which we can provide an overview of the organic farms' statuses regarding the implementation of good agricultural practices. Based on the questionnaire results, all of the farms were operating at a moderate to high level of risk for microbiological contaminations. It could be useful to implement a higher level of control activities in order to address

higher levels of risk (Kirezieva, Jacxsens et al., 2013; Kirezieva, Nanyunja et al., 2013). However, the observed operations of control activities at the farms was very low, indicating that good agricultural practices and control measures such as manure composting and water control were not implemented or applied in practice. Regarding preventive measures on lettuce farms, the microbial quality and method of composting manure as well as the source and quality of irrigation waters and washing waters could be considered of utmost importance. These findings were confirmed by the obtained microbiological results. It was demonstrated that the fertilizer control program and the water used for irrigation and washing were important factors to be controlled in the production chain of organic lettuce in order to contribute to food safety or hygiene status. The contamination of manures highlighted the need for a fertilizer control program in order to control the composting time and avoid the addition of fresh manure to the composted manure. With regards to irrigation and rinse waters, the results showed the importance of using water from safe sources. The need for consumer awareness must be emphasized because organic vegetables may not be affected by chemical contamination, but they can be contaminated with pathogens, and, for that reason, sanitization procedures should be used to avoid foodborne illnesses. The present survey indicates that the organic lettuce production chain is susceptible to microbiological safety issues and discusses the basic level at which good agricultural practices are conducive to safe produce. Moreover, assurance activities and information about their own performances were not present or were not yet elaborated, so the farms could not demonstrate that they were working correctly. Here, an important role can be played by the sector associations or the government in setting up monitoring and inspection plans.

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